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SPERMATOGENESIS IN EQUISETUM

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 158

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(WITH PLATES VII AND VIII)

Historical résumé

The cilia-bearing organs of the motile cells of plants have formed the basis of a number of researches during recent years. In the majority of cases in which the bearing of the results has been given consideration, the discussion has centered about the morphological nature of these organs, and in this discussion a very prominent place has been taken by the centrosome.

Among the earliest investigations in this field were those of STRASBURGER (**78**) on the algae. During the development of the swarm spores of *Oedogonium*, *Cladophora*, and *Vaucheria* he found that the nucleus approaches the plasma membrane, which at that point becomes thickened, forming a lens-shaped *Mundstelle*. From this grow out the cilia, and at the base of each a small refractive granule is present. A full discussion of the morphological nature of these cilia-bearing structures and an extensive comparison with those of higher plants were given in connection with a later work (**80**). The main point to be noted at this time is that STRASBURGER believed that the blepharoplasts of higher plants have been derived from such swollen *Hautschicht* organs in the algae, and that all of them are morphologically distinct from centrosomes.

DANGEARD (**17**) found a deeply staining granule at the base of the cilia in *Chlorogonium*, but did not consider it a centrosome. In

a later paper (**18**) he states that in *Polytoma* the cilia are inserted on a similar granule which is believed to be a swelling of the ectoplasm. In some cases he saw a delicate filament connecting this with another minute body at the surface of the nucleus.

In *Hydrodictyon* (TIMBERLAKE **85**) the cilia are inserted on a small body lying in contact with the plasma membrane, but independent of the latter. Protoplasmic strands join this structure with the nucleus. At the poles of the spindle during the differentiation of the spore *Anlage*, and later near the nucleus, two heavily staining granules were seen, but their origin and further history were not worked out.

In the zoospore origin of *Derbesia*, according to DAVIS (**21**), the nucleus migrates toward the cell membrane and from it many granules, which are not centrosomes, move out along radiating strands of cytoplasm to the surface of the cell, where by fusion they form a ring-shaped structure from which the cilia develop.

The development of the spermatozoid in *Chara* has been described by BELAJEFF (**2**) and by MOTTIER (**71**). Here the blepharoplast arises as a differentiation of the plasma membrane and bears two cilia. No centrosomes or *Plasmahöcker* were observed at the base of the cilia, although SCHOTTLÄNDER (**75**) had previously reported centrosomes in the cells of the spermatogenous filament.

GRIGGS (**34**) describes in a recent paper a deeply staining body at the insertion point of the cilia in the zoospore of the fungus *Rhodochytrium*. This is connected by fine cytoplasmic fibers with the nucleus. The author states that no centrosomes were observed.

In the myxomycete *Stemonitis* JAHN (**56**) has made a highly suggestive observation. During the last mitosis in the formation of the swarmers the spindle poles are occupied by centrosomes. During the anaphases the flagella of the two resulting swarmers are seen growing out directly from these centrosomes.

Among the bryophytes the blepharoplasts in *Marchantia* and *Fegatella* have received the most attention. According to IKENO (**53**) a centrosome comes out of the nucleus at each spermatogenous division in *Marchantia* and divides to two, which diverge to opposite

sides of the cell, occupy the spindle poles, and disappear at the close of mitosis. It is possible that they are included within the membranes of the daughter nuclei. After the last (diagonal) division, however, they remain in the cytoplasm as the blepharoplasts, elongating and bearing two cilia. IKENO regards these bodies as true centrosomes. He further believes that the blepharoplasts of pteridophytes and gymnosperms are derived ontogenetically or phylogenetically from centrosomes, but that all bodies called centrosomes in plants may not be homologous. In a paper appearing two years later, MIYAKE (**66**) states that although an inconstant aster, often with a dot at the focus, may appear in the spermatogenous divisions, no body like IKENO's centrosome is present, except at the last mitosis, when a body lies at each spindle pole as figured by that author. Essentially the same results were obtained in *Fegatella*, *Pellia*, *Aneura*, and *Makinoa*. MIYAKE believes that the centrosome hitherto reported in the cells of the Hepaticae is nothing but a center of cytoplasmic radiation, and inclines toward the view of STRASBURGER that the blepharoplast and the centrosome are not homologous structures. ESCOYEZ (**22**) states that in *Marchantia* and *Fegatella* two "corpuscles" appear in the spermatid mother cell in contact with the plasma membrane. These occupy the spindle poles and in the spermatids function as blepharoplasts. ESCOYEZ regards these organs as distinct from centrosomes, though their origin was not traced. Centrosomes are reported by SCHAFFNER (**74**) in all the spermatogenous divisions in *Marchantia*. After the last mitosis these behave as blepharoplasts, which are consequently looked upon as modified centrosomes. BOLLETER (**8**) found in *Fegatella* a centrosome-like body near the spindle pole at the last division and observed its nuclear origin. He believes that it is present in the earlier division also.

In the antheridium of *Riccia* LEWIS (**61**) reports centrosome-like structures in the early and diagonal divisions. These apparently arise *de novo* in the cytoplasm at each mitosis, showing no continuity through the succeeding cell generations except at the last mitosis, when they persist and become the blepharoplasts. LEWIS does not think these bodies represent centrosomes.

The most recent investigations of the blepharoplast in bryo-

phytes are embodied in two papers appearing in 1911. In the first of these WOODBURN (**95**) gives an account of spermatogenesis in *Porella*, *Asterella*, *Marchantia*, and *Fegatella*. He finds that the blepharoplast is first distinguishable as a spherical granule in the cytoplasm of the spermatid, and holds that it represents, as MOTTIER (**71**) had formerly suggested, an individualized part of the kinoplasm arising *de novo* in certain spermatogenous cells. WILSON (**93**) describes the phenomena occurring in *Pellia*, *Atrichum*, and *Mnium*. In *Mnium* and *Atrichum* the spermatogenous divisions show no centrosomes, while in *Pellia* centrospheres, and probably centrosomes, are present during the later mitoses. The origin of the blepharoplast as here described is very peculiar. In the spermatid of *Mnium* a number of bodies separate from the nucleolus and pass out into the cytoplasm where they coalesce to form a "limosphere." The nucleolus then divides into two masses, both of which pass into the cytoplasm; one functions as the blepharoplast while the other gives rise to an accessory body. In *Atrichum* the first body separated from the nucleolus becomes the blepharoplast, a second forms the limosphere, and a third the accessory body. In *Pellia* the origin of these structures was not determined. In all three plants the blepharoplast goes to the periphery of the cell and produces a threadlike structure along the plasma membrane. The nucleus then moves against this thread and the two metamorphose together to form the spermatozoid. WILSON regards the blepharoplast as "probably derived from a centrosome."

According to HUMPHREY (**49**) the blepharoplast of *Fossombronia* is first seen in the cytoplasm of the spermatid.

The early papers dealing with the spermatozoid in pteridophytes, such as those of BUCHTIEN (**9**), CAMPBELL (**12**), BELAJEFF (**1**), GUIGNARD (**35**), and SCHOTTLÄNDER (**75**), give us little or no information concerning the development of the blepharoplast. Our more definite knowledge of this subject dates from 1897, when BELAJEFF published three short papers. In the first of these (**3**) it is stated that the fern spermatozoid consists of a thread-shaped nucleus and a plasma band, with a great many cilia growing out from the latter. In the plasma band is inclosed a thin thread which arises by the lengthening of a small body seen in the sperma-

togenous cell. In the second paper (**4**) the blepharoplast of *Equisetum* is first described as a crescent-shaped body lying against the nucleus of the spermatid. This body stretches out to form the cilia-bearing thread. The third contribution (**5**) is a short account of the metamorphosis of the spermatid in *Chara*, ferns, and *Equisetum*. In all of these forms a small body elongates to form a thread upon which small *Höcker* arise and grow out into cilia. In a comparison with animal spermatogenesis, BELAJEFF here homologizes the *Körperchen* (blepharoplast) in the spermatid, the thread to which it elongates, and the cilia of the plant, with the centrosome, middle piece, and tail (perhaps only the axial filament), respectively, of the animal. The following year, in connection with a further discussion, he figured the details as made out by him in *Gymnogramme* and *Equisetum* (**6**). In *Gymnogramme* the blepharoplasts appear at opposite sides of the nucleus in the spermatid mother cell, while in *Equisetum* a single blepharoplast is first figured lying close to the nucleus of the spermatid, behaving as outlined in the earlier accounts.

One of the most interesting cases is that of *Marsilia*, first described by SHAW (**76**). According to this investigator a small granule or "blepharoplastoid" appears near each daughter nucleus of the mitosis which differentiates the grandmother cell of the spermatid. During the next division these divide but soon disappear, and a blepharoplast appears near each spindle pole. In the next cell generation (spermatid mother cell) the blepharoplast divides to two which become situated at the spindle poles in the final mitosis. In the spermatid the blepharoplast shows a small internal granule; this multiplies to several and forms a band which elongates spirally with the nucleus and bears the cilia. SHAW sees in these facts no ground for the homology of the blepharoplast and the centrosome. BELAJEFF'S paper dealing with *Marsilia* appeared in the following year (**7**). He found that centrosomes occur at the poles during all, excepting possibly the first, of the series of divisions which result finally in the 16 spermatids. After each mitosis the centrosome divides to two which occupy the poles during the next mitosis, and in the spermatid it performs the function of a blepharoplast. BELAJEFF regards this as a strong confirmation

of his theory that the blepharoplast and the centrosome are homologous structures.

In *Adiantum* and *Aspidium* (THOM **84**) the blepharoplast is described as a round body in the cytoplasm of the spermatid. It is stated that it does not act as a centrosome during division, though no figures of these stages are shown.

The most recent work dealing with the blepharoplast in pteridophytes is that of YAMANOUCHI (**97**) on *Nephrodium*. In this form there are no centrosomes in the whole life history. The two blepharoplasts, which arise *de novo* in the cytoplasm of the spermatid mother cell, take no active part in nuclear division, merely lying near the poles of the spindle. In the spermatid the blepharoplast elongates in close union with the nucleus to form the cilia-bearing band.

The first known blepharoplast in plants above the algae was discovered in *Ginkgo* by HIRASÉ (**45**) in 1894. He observed two, one on either side of the body cell nucleus, and because of their great similarity to certain structures in animal cells believed them to be attraction spheres. It was not until two years later that this investigator announced the discovery of the swimming sperm of *Ginkgo*. In 1897 WEBBER (**89**) observed the same structures, noting their cytoplasmic origin. On account of several differences existing between these bodies and known centrosomes he expressed the belief that they are not true centrosomes, but distinct organs of spermatic cells, and first applied to them the name blepharoplast. FUJII (**30, 31, 32**) gave several figures of spermatogenesis in *Ginkgo*, which agree with the accounts of HIRASÉ and WEBBER. The same subject has been dealt with more recently by MIYAKE (**67**).

In two short papers appearing in 1897, WEBBER described the blepharoplast of *Zamia* (**87, 88**), and in 1901 a very full account was published (**90**). According to this author two blepharoplasts arise *de novo* in the cytoplasm. They are surrounded by radiations up to the time of the division of the body cell, but these have no part in the formation of the spindle, which is entirely intranuclear. During mitosis the blepharoplasts, lying opposite the poles, become vacuolate and break up to many granules which unite to form the

cilia-bearing band. In this paper WEBBER gives a very extensive discussion of the morphological nature of the blepharoplast which will be referred to later.

IKENO (**51**) expressed the opinion that the blepharoplast of *Ginkgo* and the cycads is not only similar to a centrosome but is a true centrosome, a view shared by GUIGNARD (**36**). Soon after this IKENO'S full account of gametogenesis and fertilization in *Cycas* appeared (**52**). In this paper it was shown that the blepharoplasts appear in the body cell, lie opposite the spindle poles during mitosis, and break up to granules which fuse to form the spiral band in a manner similar to that described by WEBBER for *Zamia*.

Several years later the same writer published two papers dealing with the morphological nature of the blepharoplast. In the first of these (**54**) he reviews the former work on the subject and makes comparisons with analogous phenomena in animals, which he believes sustain the homologies of BELAJEFF. He points out that in *Marchantia* centrosomes are present in all the spermatogenous divisions, while in other liverworts they appear much later, and from this argues that the bryophytes show various stages in the elimination of the centrosome. He strongly reasserts his belief that blepharoplasts are centrosomes and speaks of the "Umwandlung eines Zentrosoms zu einem Blepharoplast" in the development of a spermatid into a spermatozoid. The *Hautschicht* organs of the algae are also held to be ontogenetically or phylogenetically derived from centrosomes. In the later contribution (**55**) he insists less strongly upon the morphological identity of all blepharoplasts, separating them into three categories: (1) centrosomatic blepharoplasts, including those of the myxomycetes, bryophytes, pteridophytes, and gymnosperms; (2) plasmodermal blepharoplasts, those of *Chara* and some Chlorophyceae; (3) nuclear blepharoplasts, found only in a few flagellates.

The blepharoplasts of *Microcycas* (CALDWELL **11**) appear in the cytoplasm of the body cell, often very close to each other. They are surrounded by prominent radiations and lie opposite the spindle poles through mitosis. At metaphase they have already broken up and begun the formation of the spiral band.

CHAMBERLAIN (**15**) observed in the cytoplasm of the body cell

of *Dioon* a number of very minute "black granules" which he was inclined to believe originate within the nucleus. Very soon two undoubted blepharoplasts are present, and are apparently formed by the enlargement of two of the original black granules. Very conspicuous radiations develop about them, and after mitosis they form the cilia-bearing band as in other cycads. In an earlier paper (**13**) on the homology of the blepharoplast CHAMBERLAIN expressed the opinion that it is to be regarded as a centrosome.

From the foregoing historical review it is evident that there are two general views concerning the morphological nature of the blepharoplast as seen in bryophytes, pteridophytes, and gymnosperms:

(1) The blepharoplast represents a centrosome (HIRASÉ, IKENO, BELAJEFF, GUIGNARD, SCHAFFNER, WILSON, CHAMBERLAIN).

(2) The blepharoplast is specialized kinoplasmic or cytoplasmic material but not a centrosome (STRASBURGER, WEBBER, SHAW, LEWIS, THOM, ESCOYEZ, WOODBURN).

The present study of spermatogenesis in *Equisetum* was undertaken in the hope of shedding further light upon the relative merits of these two views, and it is with particular reference to this problem that the results are given consideration in the following pages.

Materials and methods

Spores were collected in Chicago, May 15, 1911, and sown upon clean sand watered from below. These cultures were kept under ground glass in the greenhouse of the Hull Botanical Laboratory. In five weeks, a somewhat longer time than is usually necessary, sperms were swimming in large numbers.

Several killing fluids and stains were used. By far the most satisfactory results were obtained with the iron-hematoxylin of Haidenhain after a killing fluid made up as follows: bichromate of potash 2.5 gm., bichloride of mercury 5 gm., water 90 cc., freshly distilled neutral formalin 10 cc.

Description

The usual statement concerning the antheridium of *Equisetum* is that it occurs in two forms, developing in some cases like the antheridium of the eusporangiate pteridophytes, and in others from a

papillate cell as in the Filicales. The mode of development has usually been correlated with the position of the antheridium initial in the prothallium. An adequate study of antheridium development was not made in connection with the present work on *Equisetum arvense*, but of the many young antheridia examined not one was unmistakably of the latter form. Apparently any of the cells of the prothallium, especially those near the apex, are able to divide periclinally and produce antheridia of the well known imbedded type.

In the nuclei of the spermatogenous cells the chromatin has the form of a ragged network of rather close mesh, the greater part of it being accumulated in knots at the intersections. One or more conspicuous nucleoli are present. The cytoplasm during the earlier cell generations may contain many plastids in various stages of disorganization; in most cases these are no longer evident by the time the 8 or 16-celled stage has been reached, but occasionally they persist and are found in considerable numbers in the penultimate cell generation, or even in the spermatids. It is obviously necessary to select for critical study of the details of blepharoplast development those antheridia in which the plastids do not introduce an element of uncertainty.

The main point to be noted in connection with mitosis in the early cell generations is that there are present no bodies which could possibly be interpreted as centrosomes. The spindle fibers are very weakly developed and end at the poles without any signs of centrosomes, centrospheres, or asters.

The first conspicuous indication of approaching sperm formation is seen in the rounding off of the cells of the penultimate generation (fig. 1). They begin to separate at the corners and gradually draw away from each other until they are entirely free. Although the division of these cells results in the production of sperms n pairs, it becomes inaccurate to speak here of mother cells with two sperms developing in each, since the intervening walls may persist until the sperms are mature or may break down at once. By designating them "penultimate cells" this ambiguity is avoided. Their number at the time of rounding off varies greatly in different antheridia. The observed range was 64 to 512, which means that

the number of sperms per antheridium varies from 128 to 1024 (approximately). Correlated with this is a great difference in the size of the antheridia. The sperms themselves also show considerable variation in dimension, as a comparison of figs. 28 and 29 will show. The nucleus of the penultimate cell at the time of separation is in the resting condition. The cytoplasm has a very fine and uniform structure, and in most cases is entirely free from plastids or other inclusions. Vacuoles are present only very rarely.

While the penultimate cells are rounding off from one another there appears in the cytoplasm near the nucleus a very minute granule which stains intensely with iron-hematoxylin (fig. 2). Its diameter lies between 0.25 and 0.3 μ. Very faint cytoplasmic radiations extend out from it in all directions, forming a very weakly developed aster. In other cells of the same antheridium the granule is seen to be dumb-bell shaped, and in still other cells distinctly double, showing that it divides to two (figs. 3, 4). These paired bodies are the blepharoplasts. Immediately after division their diameter increases to 0.5 μ. Their radiations become more pronounced and the nucleus often becomes flattened or slightly indented at the point where they lie, as in fig. 8.

The origin of the single granule cannot be stated with certainty. When first made out it holds a position near the nuclear membrane, a fact which would suggest its nuclear origin, but no other evidence in favor of this interpretation was obtained. The nuclear membrane shows no indication of recent disturbance. Moreover, it is highly improbable that such a granule could be distinguished within the nucleus because of its small size, its similarity in staining reaction to the chromatin network, and the density of the latter. Some light may be shed upon the question by exceptional cells like that shown in fig. 5. Here are scattered through the cytoplasm many very small intensely staining bodies, a few of which occur in pairs. When first seen these granules lie in all positions with respect to the nucleus and the plasma membrane. Some of the paired granules are distinctly larger than the single ones; the pair nearest the nuclear membrane is always the largest, has the most evident radiations, and is without doubt the same structure shown in fig. 4.

In other cells of the same antheridium only this pair is present, the other bodies, if formerly present, having been resorbed.

One can hardly speak conclusively regarding all points in the history of such minute structures. The evidence at hand, however, inclines the present writer toward the belief that the original single granule, which by division gives rise to the two blepharoplasts, is in some cases one of a number which may appear *de novo* in the cytoplasm and start development.

The two blepharoplasts, which lie very close together for a little time immediately following their formation from a single body, soon begin to move apart. As they do so a very distinct central spindle develops between them, so that a faint but undoubted amphiaster is formed (figs. 6, 7). In some preparations the rays on the side toward the nucleus are somewhat heavier than the others and form a distinct cone (fig. 6). This feature is not made out in all cases. A line joining the two blepharoplasts may lie in any position with respect to the nuclear membrane, though the situation shown in fig. 6 is the most usual one. The blepharoplasts continue to separate, moving in paths close to the nuclear membrane, until they lie 180° apart (figs. 8–12). During the earlier stages of the migration the central spindle gradually fades out (fig. 8). The astral radiations persist, and when the blepharoplasts reach polar positions those on the side toward the nucleus become more distinct, being especially conspicuous when the blepharoplasts move a little distance away from the nucleus (fig. 13). They form two cones with the blepharoplasts at their apices, while the radiations extending in other directions remain very faint. The rays of the cone do not diverge from a single point on the blepharoplast, but pass out from a large portion of its surface. At this stage the blepharoplast may reach a diameter of 0.75 μ.

In the nucleus are now seen indications of the approaching mitosis which is to differentiate the spermatids. The nuclear reticulum gradually becomes coarser and eventually resolves itself into a spirem (fig. 14). While the spirem is segmenting to form the chromosomes the nuclear membrane breaks down and the fibers radiating from the blepharoplasts extend into the nuclear cavity and establish the karyokinetic figure. The spindle is extremely

weak in development, so that the relation it bears to the blepharoplasts is not always easily determined at this time. There is no question, however, that the blepharoplasts continue to occupy the poles (fig. 15), as would be expected after the situation in the immediately preceding stages. In the later stages of division many extremely fine strands are present between the daughter nuclei. Whether these are the remains of fibers passing from one blepharoplast to the other or represent the visible effect of the separation of the chromatin upon the cytoplasm was not determined. The cell plate separating the paired spermatids is very late in forming.

During the anaphases of karyokinesis a peculiar change occurs in the blepharoplasts. For a time they lose their affinity for iron-hematoxylin, so that in many preparations treated in the usual way with this stain they may be wholly indiscernable. In more deeply stained cells they appear as translucent bodies considerably larger than during the earlier phases of division (fig. 16). They are no longer solid but contain one or two large vacuoles, which give them in section the appearance of small rings. It is probable that the decrease in staining capacity is due to swelling through the absorption of water without any increase in stainable material. This condition exists only through the remainder of the division; when the sister spermatids are well rounded away from each other the blepharoplasts as a general rule stain deeply again. The vacuole or vacuoles form an irregular cavity, and the whole structure soon takes the form of an uneven ring (figs. 17, 18).

The blepharoplast now breaks up to several pieces which become arranged in a row, usually at once (fig. 19). These pieces multiply rapidly by further fragmentation and form a beaded chain extending about halfway around the nucleus (fig. 21). Fig. 20 shows a mass of these granules just beginning to draw out into a row.

It is at this beaded stage that the cilia begin to develop. From the blepharoplast granules there are seen very fine strands extending toward the periphery of the cell (fig. 21). Whether more than one of the strands, or rudimentary cilia, ever grow out from a single granule was not definitely determined, but since the cilia of the mature spermatozoid and the granules are approximately equivalent in number, it is probable that as a rule each granule gives rise

to one cilium. The further details of cilium development were not worked out.

The blepharoplast granules, which have been lying close together or in contact, now fuse to form a continuous thread. The coalescence usually begins at one end of the chain so that at certain stages it appears solid at one extremity and broken at the other (fig. 22). The union, although intimate, is not so complete that the thread is uniform in diameter throughout, even in the later stages. When the metamorphosis of the spermatid is half complete the beaded nature of the blepharoplast is clearly evident, and when the spermatozoids are mature it still shows an uneven outline.

Immediate y after the union of the granules the nucleus begins to show marked changes. It moves to one side of the cell and begins to draw out into a flattened point next to the blepharoplast (fig. 23). At this stage the nucleus and the blepharoplast lie rather close together; the relative position of the two is seen in fig. 24, which represents a portion of a similar cell viewed from the direction *a*. The nucleus continues to elongate and quickly assumes a crescentic form, while its reticulum becomes very coarse and deeply staining (fig. 25). The blepharoplast also lengthens spirally, and the two become widely separated from one another. Figs. 26 and 27 represent respectively an entire cell like that of fig. 25 viewed from the direction *a*, and a section in the plane *ab*. No connections other than the undifferentiated cytoplasm are present between the nucleus and the blepharoplast. The cilia have now increased markedly in length.

As previously noted, the mature spermatozoids vary greatly in size in different antheridia, which may be seen by comparing figs. 28 and 29. The nucleus now stains intensely with iron-hematoxylin. Its surface presents a mottled appearance, while very lightly stained sections show that its interior is quite homogeneous, with several very small vacuoles along the central region. Scattered over the nucleus, mostly along its concave face and occasionally elsewhere in the cytoplasm, are many black globules whose origin and nature were not determined. A few vacuoles are present in the cytoplasm. The blepharoplast continues its spiral growth until it has made about 1.4 turns. The nucleus makes 0.7 of a

turn, but lies parallel to the blepharoplast for 0.44 of a turn, so that the entire spermatozoid makes 1.66 turns. In all of the spermatozoids examined the direction of coiling is the same—from right to left beginning at the innermost end of the blepharoplast when the side of the cell containing the latter is turned toward the observer.

After escape from the antheridium the larger or posterior portion of the nucleus becomes extended and somewhat flattened. Both nucleus and cytoplasm absorb water and show decided enlargement, the cytoplasm, especially in the posterior portion of the spermatozoid, becoming very coarse and foamy through the great enlargement of the vacuoles. Such a mature spermatozoid fixed in the swimming condition over osmic fumes is represented in fig. 30. Exclusive of the cilia it has a length of 19.7 μ.

Discussion

The morphological nature of the blepharoplast is a topic which has been so extensively discussed by STRASBURGER, WEBBER, IKENO, and others that the present writer does not take up the subject with reference to the additional evidence afforded by *Equisetum* without risk, or even necessity, of a certain amount of repetition. In the foregoing historical résumé it was seen that the central point of the discussion has been the question of the possible morphological identity of the centrosome and the blepharoplast. Any analysis of the relationship existing between these two structures must include a consideration of the centrosome as found elsewhere in the plant kingdom, and since it has to do with a cell problem of general interest it should proceed in the light of certain phenomena occurring in the spermatogenesis in animals.

One of the earliest known centrosomes in plants was that discovered by BÜTSCHLI (**10**) in the diatom *Surirella*. It had earlier been seen by SMITH (**77**), who, however, did not recognize its true nature and termed it the "germinal dot." A full account of this centrosome was given by LAUTERBORN (**59**) in his magnificent work on the diatoms, and later by KARSTEN (**57**). It lies near the nucleus, becomes surrounded by radiations, divides and forms the central spindle of the karyokinetic figure in a very peculiar manner.

During karyokinesis it lies near the pole of the broad-poled spindle.

Centrosomes in the Sphacelariaceae have been described by STRASBURGER (**78**), HUMPHREY (**48**), and SWINGLE (**83**). In the vegetative cells of *Sphacelaria* the centrosome, according to STRASBURGER, is situated in a centrosphere at the center of an aster. Previous to mitosis it divides to two which take up positions at opposite poles. In *Stypocaulon* SWINGLE has shown that the centrosome, which lies close to the nucleus, divides, the daughter centrosomes diverging to opposite sides of the nucleus and occupying the spindle poles throughout mitosis. At all stages asters are present. SWINGLE is inclined to regard this centrosome as a permanent organ of the cell.

In the oogonium and segmenting oospore of *Fucus* FARMER and WILLIAMS (**24, 25**) described two centrospheres arising independently 180° apart. In the centrosphere they often observed several granules, but were inclined to attach no importance to them. STRASBURGER (**79**) reported definite centrosomes with asters all through karyokinesis; in the sporeling are stages which indicate that it is a dividing body. He regarded it as a permanent cell organ. In a more recent investigation YAMANOUCHI (**98**) demonstrates in the antheridium and oogonium two very definite centrosomes, which appear independently of each other, become surrounded by conspicuous asters, and occupy the spindle poles during karyokinesis. He further shows that when the sperm reaches the egg nucleus a new centrosome appears on the nuclear membrane at the spot where the sperm entered.

The centrosome of *Dictyota* has been dealt with by two investigators. MOTTIER (**69, 70**) states that in the two divisions in the tetraspore mother cell, in at least the first three or four cell generations of the sporeling and in all the vegetative cells of the tetrasporic plant, a curved rod-shaped centrosome with an aster occurs at the spindle pole. During the early phases of karyokinesis it divides, the daughter centrosomes passing to opposite poles. WILLIAMS (**91**) figures centrosomes and asters essentially like those described by MOTTIER. He also states that the entrance of the sperm causes a centrosome with radiations to appear in the egg cytoplasm.

WOLFE (**94**) found in his study of *Nemalion* that the spindle poles are always occupied, except possibly in the antheridial mitoses, by two heavily staining bodies which he considers centrosomes. They are surrounded by hyaline areas and apparently divide, but no radiations are present.

In *Polysiphonia* (YAMANOUCHI **96**) there are during the prophases of every mitosis two centrosome-like bodies in the kinoplasm at opposite poles of the nucleus. A little later the small bodies disappear, while the kinoplasm takes the form of large centrosphere-like structures without radiations. During the late anaphases these become indistinguishable. YAMANOUCHI believes that these structures are not permanent cell organs, but are formed *de novo* at the beginning of each mitosis.

In the tetraspore mother cell of *Corallina* (DAVIS **19**, YAMANOUCHI **99**) two deeply staining masses, or centrospheres, occur at opposite ends of the nucleus during the prophases of karyokinesis. They occupy the spindle poles and are surrounded by radiations. During the later anaphases they disappear and are formed *de novo* at the next division. No true centrosomes are present.

Among the fungi the best known centrosomes are those of the Ascomycetes. HARPER (**40, 41, 42, 43**) has described in the asci of *Peziza*, *Ascobolus*, *Erysiphe*, *Lachnea*, *Phyllactinia*, and other genera granular disc-shaped centrospheres surrounded by asters at the poles of the spindle. He regards them as permanent organs of the cell. GUILLIERMOND (**37, 38**) shows the presence of centrosomes and asters in several other genera. Especially interesting is the account of *Gallactinia succosa* given by MAIRE (**62**) and later by GUILLIERMOND (**39**). In the ascus of this form a single centrosome arises within the nucleus with a cone of fibers extending toward the chromatin. It divides to two which take up positions 180° apart at the nuclear membrane, at which time asters develop in the cytoplasm. FAULL (**26**) found that in *Hydnobolites* a large centrosome appears outside the nucleus during the prophases of karyokinesis. In *Neotiella* the spindle terminates in minute centrosomes with astral rays very faint or absent. In *Sordaria* he describes the centrosomes as disc-shaped while the cell is in the resting condition and round and smaller during division. The formation of the

spindle was not made out in these three forms. According to SANDS (**73**) the discoid "central body" or centrosome of *Microsphaera* divides with its aster to two which occupy the poles during karyokinesis. In *Humaria rutilans* Miss FRASER (**27**) saw at first two centrosomes lying near each other, each at the apex of a cone of fibers and surrounded by a very faint aster. These move apart and establish the spindle in the usual way. Centrosomes are also figured in *Ascobolus* and *Lachnea* (FRASER and BROOKS **29**). In *Otidea* and *Peziza vesiculosa* (FRASER and WELSFORD **28**) there are distinct centrosomes and asters. The figures given in this paper indicate that division of the centrosome occurs in the latter species. In a recent contribution CLAUSSEN (**16**) figures centrosomes with weakly developed asters in *Pyronema*. The origin of the spindle is not shown.

The first centrosome described in the liverworts was that of *Marchantia* by SCHOTTLÄNDER in 1893 (**75**). According to this observer the centrosome in the spermatogenous cells divides during the anaphases of mitosis, so that each daughter nucleus is accompanied by two. In the gametophytic cells certain minute bodies with radiations at the poles of the elongated nucleus and of the spindle are believed by VAN HOOK (**86**) to represent centrosomes.

Pellia has been the subject of four investigations dealing with the centrosome. In 1894 FARMER and REEVES (**23**) gave an account of mitosis in the germinating spore. They reported two centrospheres at opposite sides of the nucleus with conspicuous radiations but no true centrosomes. The centrospheres occupy the spindle poles and disappear during the telophases of division. DAVIS (**20**) studied the same mitoses and obtained similar results. He states, however, that the centrospheres fade out somewhat earlier. The account given by CHAMBERLAIN (**14**) agrees with these in the essential features. The structures are very distinct in the first mitosis but become less so in the succeeding ones. The most recent work is that of GREGOIRE and BERGHS (**33**). By using improved methods these investigators have found that neither in the resting cells nor during mitosis are there centrospheres or central corpuscles. The centrospheres described by other writers are

shown to be appearances due to the intersection of the very numerous astral radiations at a common point or region. The achromatic figure is derived entirely by the rearrangement of the cytoplasmic network.

As the centrosome becomes more widely known it becomes increasingly difficult to formulate for it any adequate definition. There is scarcely a single attribute common to all true centrosomes; nevertheless there are in general certain features which are fairly characteristic of them as they appear in plants and animals, most prominent among which are the position at the spindle poles with all that this implies, the possession of an aster, and the division to form daughter centrosomes. Because of many exceptions no one of these by itself will definitely determine the morphological nature of a structure possessing it, but when all of them are present we can no longer doubt that we are dealing with a true centrosome.

In a survey of the cilia-bearing structures of bryophytes, pteridophytes, and gymnosperms it is seen that in general the centrosome-like characteristics of the blepharoplast become less and less evident in passing upward through these groups, while the phenomena connected with the bearing of cilia become increasingly prominent. In the bryophytes the conflicting accounts leave us in some doubt concerning the early history of the blepharoplast, but in some cases at least it appears that centrosomes exist through several cell generations and after the last mitosis function as blepharoplasts. In those forms which show them only during the last division they occupy the spindle poles and behave as typical centrosomes. In the spermatids each simply elongates and bears two cilia. In the Filicales, as shown by YAMANOUCHI'S work on *Nephrodium*, the blepharoplast is limited to the last mitosis and does not exhibit the characters of a centrosome, having no division, no radiations, and only occasionally occupying the pole of the spindle. It elongates in intimate union with the spermatid nucleus and bears many cilia. In the gymnosperms the blepharoplast, although surrounded by prominent radiations, appears to play little or no active part in mitosis. In its subsequent behavior it differs widely from the blepharoplasts of the bryophytes and Filicales. After enlarging it becomes vacuolate and breaks up into many fragments, which

arrange themselves in a row and coalesce to form the cilia-bearing band.

The peculiar interest of the phenomena in *Equisetum* is here evident. Although limited to a single mitosis in the antheridium, the blepharoplast retains in its activities the most unmistakable evidences of a centrosome nature, and at the same time shows a metamorphosis strikingly like that in the cycads. In thus combining the main characteristics of true centrosomes with the peculiar features of the most advanced blepharoplasts, it reveals in its ontogeny an outline of the phylogeny of the blepharoplast as it is seen developing through bryophytes, pteridophytes, and gymnosperms, from a functional centrosome to a highly differentiated cilia-bearing organ with very few centrosome resemblances. In *Marsilia* the same pronounced centrosome behavior is shown through at least three cell generations, and in the formation of the cilia-bearing band the cycad situation is foreshadowed, though not to the marked degree seen in *Equisetum*. To the present writer these facts seem to constitute conclusive evidence in favor of the theory advanced by BELAJEFF and by IKENO, that the centrosome has gradually assumed the function of bearing cilia, at the same time losing the usual properties of a centrosome.

The points brought out in such a review are especially suggestive in connection with the conclusions to which WEBBER has been drawn by his studies on *Zamia* (**90**). This investigator emphasizes very strongly the view that the blepharoplast is a distinct organ functioning only as a cilia-former, and urges several objections to its centrosome nature. He points out that it differs from known true centrosomes in not being at the center of an aster at the poles and having no connection with spindle formation, in being limited to a single cell generation, in its great size, in its fragmentation, in its growth into a band, in its function of bearing cilia as far as plant centrosomes are concerned, and in its behavior in fertilization. Although the blepharoplasts of other plant groups are discussed, it appears that these conclusions must have been formulated largely through a consideration of the cycad situation. When the blepharoplast is regarded as an organ developing progressively through bryophytes, pteridophytes, and gymnosperms,

and is treated in the light of analogous phenomena in animals, much of the apparent force of these objections is removed.

In *Marsilia*, as BELAJEFF indicates in his fig. 7 (**7**), and in *Equisetum*, the blepharoplasts are surrounded during the early stages by asters, though these are very weakly developed. When they separate there appears a central spindle, forming with the asters an amphiaster so characteristic of animal cells. In *Equisetum* the radiations persist during the divergence of the blepharoplasts to opposite sides of the cell, and those on the side toward the nucleus remain as the achromatic portion of the karyokinetic figure. The weakness of the other rays or their failure to remain seems to be a matter of secondary importance in the light of spindle-forming activity of this sort. Furthermore, the figures given by zoologists indicate that the occurrence of an aster about the centrosome at the spindle pole is by no means universal in animal cells. In discussing this phase of the question IKENO (**54**) cites the work of MEVES and KORFF (**65**) upon the myriopod *Lithobius forficatus*, in which the spermatocyte centrosomes lie at a considerable distance from the spindle poles during mitosis. The figures given by MEVES and KORFF are strikingly like those of *Ginkgo* (HIRASÉ) and *Cycas* (IKENO).

It is true that the blepharoplast is, as a rule, limited to a single mitosis, but here we must remember the case of *Marsilia* where it is present during three, possibly all four, of the spermatogenous divisions, and also certain liverworts in which a similar condition has been reported. WEBBER accounts for the occurrence of blepharoplasts in all the spermatogenous cell generations in *Marsilia* by considering the latter potential spermatozoids, and thus regards the fact that they appear *de novo* in each cell generation only to disappear at the close of division as a support to his theory of the independent nature of the blepharoplast. If the cells between the central cell of the antheridium and the final spermatids are held to be potential spermatozoids, we should expect, as WEBBER points out, blepharoplasts or their rudiments to be present occasionally. Although these ideas are in accord with the conception of the blepharoplast as an organ *sui generis*, at the same time it does not seem to the present writer that they offer any necessary argument

against its centrosome nature, especially since such "rudiments" as are seen in the spermatogenous cells of *Marsilia* and probably certain liverworts are so remarkably centrosome-like. Moreover, many true centrosomes appear *de novo* in each successive cell generation only to disappear at the close of mitosis.

If the centrosome be an organ which has been practically eliminated from higher plants, we should not be surprised to see it retained, if at all, in different degrees in different plants, and in those cells in which it performs an important biological function, as other workers have suggested. WEBBER's statement that no known plant centrosome has the function of bearing cilia is no longer without a possible exception, since JAHN (**56**) has seen the flagellum of the swarmer of *Stemonitis* growing out from the centrosome during mitosis, exactly paralleling what HENNEGUY (**44**) observed in an insect. That the bearing of cilia is the function which is to be held accountable for the retention of the centrosome in spermatogenous cells seems highly probable. After having lost the usual functions of a centrosome we might well find it appearing still later, in the spermatid itself, as WOODBURN (**95**) believes it does in certain liverworts. BELAJEFF's view concerning the presence of these structures only in the spermatogenous cells is that every cell has its definite "dynamic center," but only in these cases is a staining substance present.

That growth into a band or thread does not deny the centrosome nature of an organ is shown by the great bodily elongation of the inner centrosome in the spermatozoon of *Helix* (KORFF **58**) and certain elasmobranchs (SUZUKI **82**, MOORE **68**). The rodlike centrosome of *Dictyota* and the discoid one of certain ascomycetes constitute a further argument against allowing the character of shape to enter into the definition of the centrosome.

Thus from the standpoint of the theory stated in the foregoing pages, the occurrence of secondary peculiarities developed in connection with cilia-bearing in the cycads and certain pteridophytes, such as large size, fragmentation, and growth into a band, does not distinguish the blepharoplast from the centrosome. This is emphasized by the fact that the first two of these features do not occur in the blepharoplasts of bryophytes and most pteridophytes,

but begin to appear in other members of the latter group, combined with earlier stages in all essential points centrosome-like.

Both WEBBER and STRASBURGER have pointed out that the blepharoplast, since it remains behind in the cytoplasm of the egg and does not meet the female nucleus, is inactive in fertilization, while in animals the centrosome brought into the egg by the spermatozoon plays a very important rôle in fertilization and in the first cleavage mitosis. They advance this as a further evidence that the blepharoplast and the centrosome are not homologous. We have seen that as the blepharoplast has become more and more highly differentiated in relation to the bearing of cilia, it has gradually lost the characters which would serve to mark it as a centrosome. The disappearance of activity during fertilization along with the other usual centrosome functions would be expected, if, indeed, the sperm centrosome of plants ever did take any active part in this process. In *Nephrodium* (YAMANOUCHI **97**) and probably many other pteridophytes and bryophytes the entire spermatozoid enters the egg nucleus, but it is highly improbable that the presence of the blepharoplast in these cases is necessary to fertilization. On the other hand, we cannot yet certainly conclude that a structure is entirely passive in fertilization merely because it does not reach the female nucleus or produce other striking visible effects. In any case it should be remembered that function is not that upon which we can base homology.

In denying the identity of the blepharoplast and the centrosome STRASBURGER (**80**) derives the blepharoplasts of bryophytes, pteridophytes, and gymnosperms from the thickened *Hautschicht* organs of algal swarm spores and gametes. This theory appears to have the support of current conceptions of phylogeny, but it leaves the remarkable behavior of the liverwort, *Marsilia*, and *Equisetum* blepharoplasts to be accounted for. That the *Hautschicht* organ seen in algae should assume, during the course of evolution, such centrosome-like characters, adding them at the earlier end of its life history, seems more difficult of comprehension than the theory stated in the foregoing pages—that the centrosome has gradually taken on the cilia-bearing function.

Through his work on *Marchantia* IKENO was led to state a view which might appear to lessen the contrast between the above two theories. He pointed out (**54**) the resemblance between the elongation of the blepharoplast along the plasma membrane of the *Marchantia* spermatid and the formation of the thickened portion of the *Hautschicht* in the algae as described by STRASBURGER and others, and concluded that this thickening has almost without doubt been derived from a centrosome ontogenetically or phylogenetically, that it is the metamorphosis product of a centrosome. His belief that the basal body in the swarm spore of *Hydrodictyon* is to be accounted for in a similar way was strengthened by the fact that TIMBERLAKE (**85**) observed what were evidently centrosomes at the poles of the spindles giving rise to the spore *Anlage*. In his later paper (**55**) IKENO is less inclined to include the algal *Hautschicht* organs in the same morphological category with the blepharoplasts of the higher plants, but places them in a class apart—"plasmodermal blepharoplasts."

In the light of our limited knowledge of the history of the blepharoplasts in algae it seems wisest to make this disposition of them for the present. Otherwise we should be compelled to assume their homology with those of the higher groups from which they differ so widely in origin, appearance, and general behavior. Since we can no longer remain in doubt concerning the centrosome nature of the blepharoplast of higher plants, this assumption would mean that the alga blepharoplast has lost all centrosome properties and now arises in the motile cell itself in a very modified manner, making it farther advanced in this respect than those of the higher groups, which we can hardly regard as probable. Before any final judgment can be rendered on this question more data must be gathered from the algae themselves, from those forms which show both centrosomes and blepharoplasts in their life histories.

The researches of MOORE (**68**), MEVES (**63, 64**), KORFF (**58**), PAULMIER (**72**), and several others have established beyond question the fact that the centrosome (or centrosomes) of the animal spermatid plays an important rôle in the formation of the motor apparatus of the spermatozoon, the axial filament of the flagellum

growing out directly from it. HENNEGUY (44) even observed cilia attached to the centrosomes of the karyokinetic figure in the spermatocyte of an insect.

In comparing the structures of the plant spermatozoid with those of the animal spermatozoon, BELAJEFF (5) regarded the blepharoplast, the thread to which it elongates, and the cilia of the former as homologous with the centrosome, middle piece, and tail, respectively, of the latter. The blepharoplast of *Chara* is included in this comparison in spite of the apparent difference in its mode of origin. STRASBURGER (80), although agreeing that the body at the base of the flagellum of the animal sperm is a centrosome, homologized only the axial filament of the flagellum with the blepharoplast. This comparison leaves both the cilia of the plant spermatozoid and the centrosome of the animal spermatozoon without counterparts, though a complete homology of this sort is by no means a necessity. The behavior of the centrosomes in the spermatid of *Helix* (KORFF 58) has made it evident that the axial filament of the flagellum is not a differentiation of the cytoplasm, starting at the centrosome, but is made up of the centrosome substance itself. Thus in comparing the blepharoplast to the axial filament its centrosome relationship is not entirely avoided. In a discussion of this question E. B. WILSON (92) regards the work of SHAW and BELAJEFF on *Marsilia* as establishing beyond question the identity of the blepharoplast and the centrosome. He considers the comparison of BELAJEFF as justified and concludes that "the facts give the strongest ground for the conclusion that the formation of the spermatozoids agrees in its essential features with that of the spermatozoa. . . . ."

The deeply staining bodies at the base of the flagella in other ciliated animal cells have also been investigated for further light upon this problem. That they correspond to centrosomes has been rendered highly probable by the work of HENNEGUY (44) and LENHOSSEK (60), while STUDNICKA (81) has obtained evidence apparently in favor of a contrary interpretation. This question must remain with others for further researches to clear up.

In the meantime it should be borne in mind that whatever interpretation is finally put upon the cilia-bearing structures of any

plant or animal group, it must not be forced upon those of all other groups. Since homologies are not determined by function, there is no necessity for expecting all of these organs to belong to the same morphological category. It is in the algae that the blepharoplast of plants at present stands most in need of elucidation. In the bryophytes, pteridophytes, and gymnosperms there can now remain no question that the blepharoplasts are all homologous structures, and that they are, to use IKENO's expression, "ontogenetically or phylogenetically centrosomes."

Summary

1. In the early mitoses in the spermatogenous tissue of *Equisetum* there are no centrosomes, centrospheres, or asters.

2. A minute granule, surrounded by a weakly developed aster, appears in the cytoplasm near the nucleus in each of the cells of the penultimate generation. This granule divides to two, which become the blepharoplasts.

3. The two blepharoplasts, each with its aster, diverge to opposite poles of the nucleus. During the early stages of separation a distinct central spindle develops, so that an amphiaster is present.

4. The astral rays on the side toward the nucleus form two cones of fibers which, when the nuclear membrane breaks down, become the achromatic portion of the karyokinetic figure. The blepharoplasts occupy the poles.

5. During the anaphases and telophases of karyokinesis the blepharoplast enlarges, becomes vacuolate, and breaks up to a number of pieces. After further fragmentation these unite to form the cilia-bearing thread.

6. In the metamorphosis of the spermatid the nucleus and blepharoplast elongate spirally side by side, but have no connection other than that afforded by the undifferentiated cytoplasm.

7. The activities of the blepharoplast in *Equisetum*, taken together with the behavior of recognized true centrosomes in plants and analogous phenomena in animals, are believed to constitute conclusive evidence in favor of the theory that the blepharoplasts of bryophytes, pteridophytes, and gymnosperms are derived ontogenetically or phylogenetically from centrosomes.

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EXPLANATION OF PLATES VII AND VIII

All figures were drawn at the level of the table with the aid of an Abbé camera lucida under a Zeiss apochromatic objective 2 mm. N.A. 1.40, with compensating ocular 18. They have been reduced one-third in reproduction, and now show a magnification of 2533 diameters.

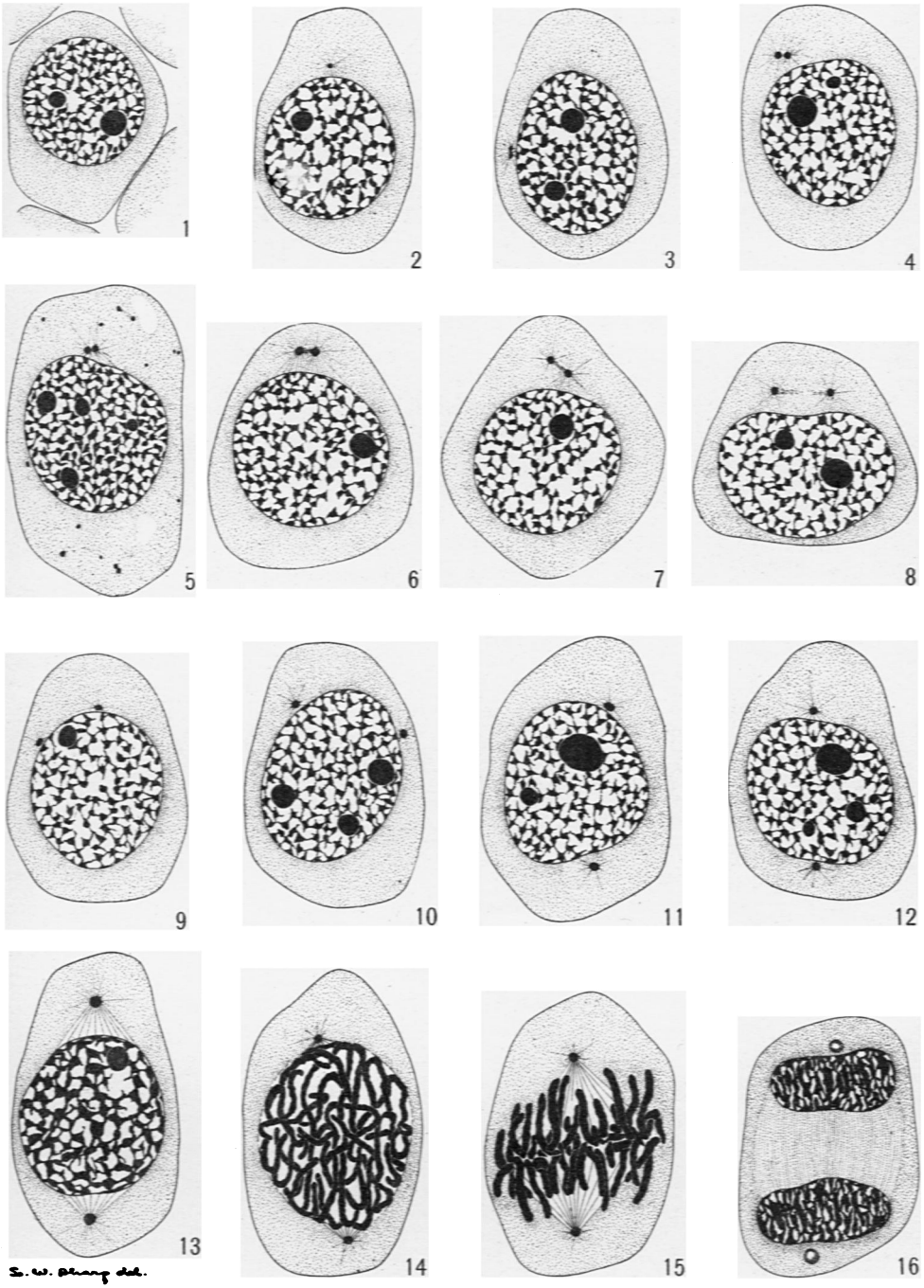
PLATE VII

FIG. 1.—Cell of penultimate generation rounding off.

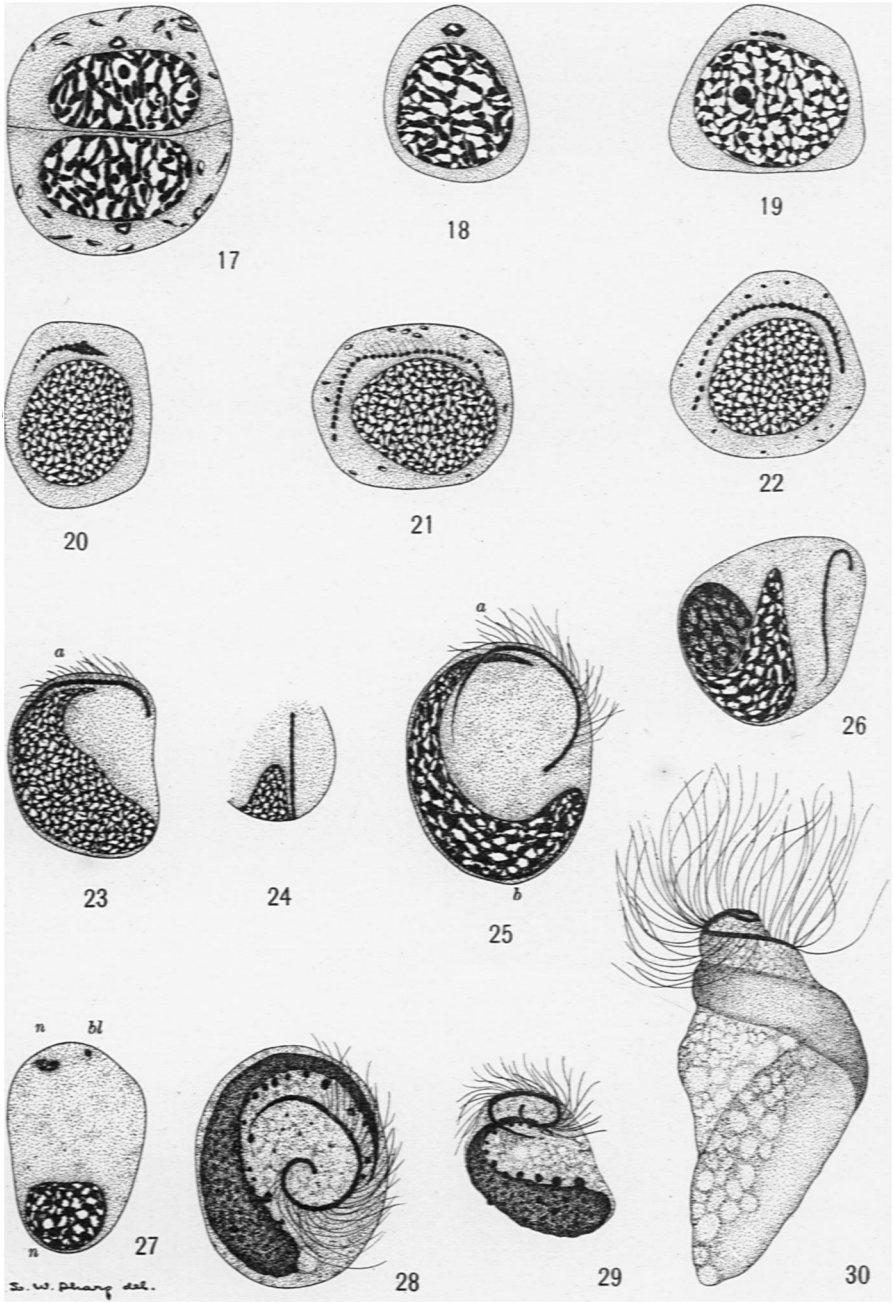
FIG. 2.—Deeply staining body with faint aster present in cytoplasm.

FIG. 3.—Division of small body in cytoplasm.

FIG. 4.—Two blepharoplasts formed by division of the original body.



SHARP on EQUISETUM



SHARP on EQUISETUM

FIG. 5.—Blepharoplasts at upper side of nucleus; other single and paired bodies present in cytoplasm; exceptional condition.

FIG. 6.—Blepharoplasts beginning to separate; central spindle present; the radiations on the side toward the nucleus form a distinct cone.

FIG. 7.—Later stage; no cone of rays present.

FIG. 8.—Still later stage; central spindle fading out.

FIGS. 9–12.—Stages in the divergence of the blepharoplasts.

FIG. 13.—Blepharoplasts lying at a greater distance from nucleus; the radiations on the side toward the nucleus form two well marked cones; the chromatin network becoming coarser.

FIG. 14.—Spirem stage: nuclear membrane beginning to break down; astral rays much shorter.

FIG. 15.—Late prophase: the spindle fibers have been formed from the radiations of the blepharoplasts, which occupy the poles.

FIG. 16.—Telophase: blepharoplasts have enlarged and become vacuolate.

PLATE VIII

FIG. 17.—Pair of spermatids differentiated: blepharoplasts have the form of irregular rings; plastids present in cytoplasm.

FIG. 18.—Spermatid: blepharoplast beginning to fragment.

FIG. 19.—Blepharoplast broken up to several pieces.

FIG. 20.—Granules formed by fragmentation of blepharoplast beginning to draw out into a row; nucleus again in resting condition.

FIG. 21.—Blepharoplast granules arranged in a long row; cilia beginning to grow out from them; plastids present.

FIG. 22.—Blepharoplast granules fusing at right end of chain; still separate at left end; degenerating plastids in cytoplasm.

FIG. 23.—Blepharoplast now a continuous thread; cilia partially developed; nucleus beginning its metamorphosis.

FIG. 24.—Portion of a similar cell viewed from the direction *a*, showing proximity of nucleus and blepharoplast; cilia not drawn.

FIG. 25.—Later stage: nucleus and blepharoplast have elongated spirally; chromatin network very coarse.

FIG. 26.—Entire cell similar to that of fig. 25 viewed from the direction *a*, showing independence of nucleus and blepharoplast; cilia not drawn.

FIG. 27.—Section of similar cell in plane *ab: n*, nucleus; *b*, blepharoplast; cilia not drawn.

FIG. 28.—Mature spermatozoid still in antheridium: the blepharoplast makes 1.4 turns, the nucleus 0.7 of a turn; deeply staining globules in cytoplasm near nucleus.

FIG. 29.—Smaller spermatozoid in another antheridium, viewed from a different direction.

FIG. 30.—Spermatozoid fixed in the swimming state over osmic fumes: the dark spiral band bearing the cilia is the blepharoplast; the lighter, homogeneous portion the nucleus; the vacuolate portion the cytoplasm; length exclusive of cilia, 19.7 μ.